Open Access
J. Glob. Innov. Agric. Sci., 2023, 11(3):333-340
ISSN (Online): 2788-4546; ISSN (Print):2788-4538
DOI: https://doi.org/10.22194/JGIAS/11.1120
http://www.jgiass.com

Compatibility of selected synergist with Diamides and Profenophos in controlling shoot and fruit borer (*Leucinodes orbonalis*) infesting brinjal in two localities of Odisha

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Mixed function oxidases groups of enzymes in insects play an established role in detoxification of insecticides and xenobiotics. It is hard to get rid of *Leucinodes orbonalis* from brinjal because of typical feeding habit of the pest and evolution of high degree of resistance to all the newly evolved insecticides In the present study six chemicals i.e. two synergists (Pipernyl butoxide, Diethyl malate), two antioxidants (Hydroquinone, N-Propagyloxy phthalimide) and two plant oils (Pongamia oil, *Callophyllum inophyllum* oil) were evaluated for their synergistic activity against chlorantraniliprole, flubendiamide and profenophos. In two populations of*L. orbonalis* collected from two diversified insecticide used area (Bhubaneswar, Angul) of Odishaduring the Kharif 2021-22. Pipernyl butoxide (PBO) applied at 10 µg per larvae with the help of Hamilton glass syringe (Hamilton Company) with three insecticides, showed highest synergistic ratio. Angul population showed higher synergistic ratio (10.22, 25.00, 33.33) as compared to Bhubaneswar population 2.33, 3.50 and 4.70. After PBO, Diethyl malate (DEM) and N-Propagyloxy phthalimide (PP) showed second and third highest synergist ratio in both Angul and Bhubaneswar, while both the plant oils and Hydroquinone were found to be insignificant.
Keywords: Mixed function oxidases, Detoxification, Insecticides, Xenobiotics, *Leucinodes orbonalis*, Resistance evolution, Synergistic ratio, plant oils.

INTRODUCTION

According to the National Horticulture Database, in 2017–18, the area under brinjal production was 1.17ha, with production of 20.13 lakh tonnes and productivity of 17.07 mt/ha. (Paul *et al.*, 2022). %%. In vegetable production, India occupies the second largest position after China. Brinjal is prone to attack by many insect pests, the most important of which is the fruit and shoot borer for which resistant cultivars has not been identified and thus, it causes significant losses of 60-70 %. The losses caused by this pest are reported by several workers and ranges from 55.66 %to 80 %% or even more 48.30 %% (Singh *et al.*, 2018). In severe infestations it causes up to 70 % yield loss of fruits in West Bengal (Singh *et al.*, 2018). Brinjal fruit and shoot borer (*Leucinodes orbonalis* Guenée) is difficult to control by insecticidal spray as it is an internal feeder and resistant to conventional insecticides. The P_{450} enzymes (sub group of Mixed function oxidases enzyme), found in insects, have a wide range of functions, including hormone synthesis, chemical metabolism, and detoxification. Insects typically carry around 100 P_{450} genes that code for different P_{450} enzymes, which are regulated through induction and mutations for adaptation and resistance. The diversity of P_{450} genes allows insects to respond to various chemical stimuli and maintains their evolutionary survival. (Feyereisen, 1999) Keeping this in view the present investigation has been undertaken to evaluate the effectiveness of few synergist and effect of

Tripathy, M.K., S. Khuntia, S.S. Dash, S. Quadri and N. Singh. 2023. Compatibility of selected synergist with Diamides and Profenophos in controlling shoot and fruit borer (*Leucinodes orbonalis*) infesting brinjal in two localities of Odisha. Journal of Global Innovations in Agricultural Sciences 11:333-340.
[Received 20 Jul 2023; Accepted 15 Sep 2023; Published 30 Sep 2023]

sublethal doses of some commonly used insecticides on Mixed function oxidases (MFOs) enzymatic systems of this pest in larvae collected from two different agroclimatic regions of the state.

MATERIALS AND METHODS

The research work was carried out in the toxicology laboratory of Department of Entomology, College of Agriculture, OUAT, and Bhubaneswar during the year 2021-22. The materials used for the study and methods adopted are given here under;

***Test insect*:** Brinjal shoot and fruit borer, *L. orbonalis* (Lepidoptera: Crambidae) population were collected from brinjal growing region of Angul (Karatapata) and Khordha (Uttara) districts of Odisha.

Collection of test insect: The 3rd instar larva of *L. orbonalis* were collected from the infested fruits collected from Angul and Bhubaneswar of the state. The larvae were kept inside a plastic jar with perforated lid or covered with net cloth. Cut brinjal fruits or potatoes were provided in the jar as a source of food to the larvae. Toxicity tests were further performed after putting the larvae into different jars.

***Source of food for test insect*:** Brinjal and Potatoes were collected from nearby vegetable market and research plot. Adequate food (10g per larvae) was provided to the test insect for their survival. *L.orbonalis* culture was maintained on potato cut pieces inside a plastic jar at 27ºC and 70% Relative humidity in the laboratory Each jar was covered with a piece of net cloth that was secured around the rim using rubber bands. Food (fresh cut piece of potatoes) was replaced every day till testing.

***Serial dilution of insecticides*:** The chemicals used are commercial grade formulations of chlorantraniliprole, flubendiamide and profenofos. The test concentration of the insecticides was prepared from the commercial formulations by adding required quantity of acetone. The dilutions were made using acetone as a solvent. Different concentrations of test insecticides were prepared by serial dilutions of the respective test insecticide. The insecticides were measured to dispense 1 µg/Larvae which was further diluted to $1x10^{-3}$, $5x10^{-4}$, $1x10^{-4}$, $5x10^{-5}$, $1x10^{-5}$, $5x10^{-6}$, $1x10^{-6}$ µg/larvae. All the serial dilutions were taken for testing. Correction factor was considered as per marketable strength of different insecticides.

***Bioassay Technique*:** The insecticides were applied topically to the dorsal thoracic segments of third and fourth instar larvae using Hamilton hand micro applicator. 36 treated larvae were released in a container after application of dose with net cloth sealed on mouth containing fresh untreated cut piece of potato and allowed to feed. Mortality count was recorded in 24 hrs, 48 hrs and 72 hrs. Bioassay was carried out to determine toxic levels of insecticide treatments ranging mortality from 20-80%. For control, acetone alone was used. Corrected mortality was computed by using Abbot's formula (Abbot 1925).

***Synergist*:** Details of insecticides and chemicals tested for synergistic activity is given in Table 1.

They were prepared by weighing the required chemical and dissolved in acetone and nontoxic dose was finalised (Table 2). The nontoxic doses of each synergist were fixed as per Leong *et al.*, 2019.Synergist were applied topically 15

Table 1. Characteristics of the chemicals used in the present study.

Sr.	Name	Type	Group	Chemical formula
1	Chlorantraniliprole 18.5% SC	Insecticide	Antranillic acid diamides	$C_{18}H_{14}BrCl_2N_5O_2$
2	Flubendiamide 39.35% SC	Insecticide	Pthallic acid diamides	$C_{23}H_{22}F_7IN_2O_4S$
3	Profenofos 50% EC	Insecticide	Organophosphate	$C_{11}H_{15}BrClO_3PS$
4	Piperonyl butoxide	Synergist (MFO inhibitor)	Methylenedioxy phenyl	$C_{19}H_{30}O_5$
5	Diethyl maleate	Synergist (Glutathion inhibitor)	-	$C_8H_{12}O_4$
6	Hydroquinone	Synergist (Antioxidant)	Benzenediol	$C_6H_6O_2$
7	N-(Propargyloxy)phthalimide	Synergist (Esterase hydrolase inhibitor)	Dicarboximide	$C_{11}H_7NO_3$
8	Pongamia oil	Plant alkaloid	Furanoflavonol (Karanjin)	$C_{18}H_{12}O_4$
9	*Callophyllum inophyllum* oil	Plant alkaloid	Pyranocoumarin	$C_{23}H_{27}O_5$

Table 2. Determination of non-toxic doses of different synergists alone on both the population collected during *Kharif* 2021-2022.

Synergist	Doses (µg/ insect)	Bhubaneswar		Angul	
		No. of dead /No. dosed	Corrected mortality %	No. of dead /No. dosed	Corrected mortality%
PBO	100	18/24	17.17	18/24	17.17
	50	11/24	10.10	10/24	9.09
	40	6/24	5.05	6/24	5.05
	30	4/24	3.03	3/24	2.02
	20	2/24	1.01	2/24	1.01
	10	1/24	0.00	1/24	0.00
DEM	100	16/24	15.15	15/24	14.14
	50	6/24	5.05	6/24	5.05
	45	4/24	3.03	4/24	3.03
	30	3/24	2.02	3/24	2.02
	25	2/24	1.01	2/24	1.01
	20	1/24	0.00	1/24	0.00
Hydroquinone	50	9/24	8.08	7/24	6.06
	40	4/24	3.03	3/24	2.02
	30	3/24	2.02	2/24	1.01
	25	2/24	1.01	2/24	1.01
	15	1/24	0.00	1/24	0.00
N-(Propagyloxy) phthalimide	50	12/24	11.11	10/24	9.09
	40	7/24	6.06	6/24	5.05
	25	4/24	3.03	3/24	2.02
	20	2/24	1.01	2/24	1.01
	15	2/24	1.01	2/24	1.01
	10	1/24	0.00	1/24	0.00
Pongamia oil	50	14/24	13.13	13/24	12.12
	40	9/24	8.08	8/24	7.07
	25	6/24	5.05	5/24	4.04
	20	4/24	3.03	3/24	2.02
	15	2/24	1.01	2/24	1.01
	10	1/24	0.00	1/24	0.00
Callophyllum inohyllum oil	150	10/24	9.09	9/24	8.08
	100	7/24	6.06	6/24	5.05
	55	5/24	4.04	4/24	3.03
	50	3/24	2.02	3/24	2.02
	45	2/24	1.01	2/24	1.01
	40	1/24	0.00	1/24	0.00
Acetone alone		1/24	0.00	1/24	0.00

minutes prior to pesticide application. Synergistic ratio was computed with the following formula.

$$\text{Synergistic Ratio} = \frac{\text{LD50 of insecticide alone}}{\text{LD50 of insecticide + Synergist}}$$

***Mixed function oxidase enzyme assay*:** Mixed function oxidase was assayed to identify their roles in imparting resistance. Enzyme determination methodology was as per Kranti (2005) and partially modified in our laboratory as per requirement as detailed below.

***Preparation of buffer (100Mm Phosphate buffer, pH 7.0)*:** Prepared by dissolving 8.89 g of dibasic sodium phosphate (MW=177.99) in 500ml of distilled water. 7.8 g of Potassium dihydrogen orthophosphate (MW=136.09) in 500ml of distilled water was added and pH was adjusted to 7 with the help of Labman auto digital pH meter.

***Preparation of homogenization and assay buffer*:** Phosphate buffer (100 mM, pH 7.0) of 500 ml was taken along with 0.186g of 1 mM of EDTA (ethylene diamine tetra acetic acid)

(MW: 372.24 g/m), 0.087g of 1mM PMSF (phenyl methyl sulfonyl fluoride) (MW:174.19g/m), 0.152g of 1mM PTU (Phenyl thiourea) (MW:152.22 g/m) and 20% glycerol. Weight of all the chemicals was taken with a high precision analytical balance.

Preparation of enzyme homogenate**:** Six larvae weighing 200-250 mg were used and phosphate buffer (pH 7) was added at the rate of 1ml per 5 mg of body weight for homogenisation. Homogenisation was done by all glass potter Potter-*Elvehjem* tissue *homogenizer*. The homogenate was first centrifuged at 4000 rpm for 20 min and the supernatant was again homogenised at 10000 rpm for 20min with the help of Eppendorf centrifuge 5430R machine. Both homogenization buffer and sodium dithionate were taken in control except the enzyme homogenate.

Determination of Cytochrome b_5 **:** To determine the amount of cytochrome b5 in the reduced state, a 3 ml enzyme solution is pipetted into a quartz cuvette. Then, 10 mg of sodium dithionite, a reducing agent, is added to the cuvette and mixed well. The mixture is incubated for 2 minutes at room temperature. Then the absorbance of the mixture is measured at 424 nm and 409 nm using a spectrophotometer. The difference in absorbance is calculated. Using the given extinction coefficient difference of 184 cm-1mM-1, the concentration of cytochrome b_5 is determined by dividing the absorbance difference by the product of the extinction coefficient and the path length. This provides an estimation of the amount of cytochrome b_5 present in the solution 184 cm^{-1} mM^{-1}.

$$\text{Cytochrome b5 } (\mu\text{M}) = \frac{(ABS\ at\ 424 - ABS\ at\ 409) \times 1000}{184}$$

Production of carbon monoxide and bubbling**:** For carbon monoxide production, 12 gm of oxalic acid (MW: 126 g/m) was mixed with 200 ml of distilled water, after oxalic acid crystals get dissolved properly then 10ml conc. sulphuric acid (H_2SO_4) was poured with care. Then the solution was kept on top of heater until it starts to boil. The gas collected was further passed through NaOH solution (20 g NaOH in 200 ml distilled water) for removing CO_2 and finally carbon monoxide was collected which was pure in form and used with help of nozzle to bubble inside the enzyme homogenate before assay.

Determination of Cytochrome P_{450}/P_{420}: 3 ml of reduced enzyme was pipetted out into 4 ml quartz cuvette and 10 mg of sodium dithionate was added, then it was transferred for carbon monoxide bubbling for 1-2mins into the glass test tube. The tube containing enzyme was covered with aluminium foil to avoid light.

Then the enzyme content was transferred into quartz cuvette and placed inside spectrophotometer (Model-Systronics136) The difference in absorbance between 450nm and490nm was used to calculate the cytochrome P_{450} using extinction co-efficient difference of 91cm^{-1} mM^{-1}. The difference in absorbance between 420nm and 490nm was used to calculate the cytochrome P_{420} content using extinction co-efficient difference of 110$cm^{-1}mM^{-1}$.

$$\text{Cytochrome P450 } (\mu\text{M}) = \frac{(\text{ABS at } 450 - \text{ABS at } 490) \times 1000}{110}$$

$$\text{Cytochrome P420 } (\mu\text{M}) = \frac{(\text{ABS at } 420 - \text{ABS at } 490) \times 1000}{91}$$

Protein estimation was carried out as per Lowry *et al.*, (1951)

The sub-lethal dose (LD_{30}) of insecticides were applied topically by means of a Hamilton micro-applicator to 30/40 mg larvae and activity of MFOs was determined at 24 , 48 and 72 h. interval in each case. Activity ratio was computed by comparing the activities of enzyme found in treated and untreated insects at above specified intervals by using formula.

$$\text{Activity ratio} = \frac{Enzyme\ activity\ in\ treated\ insect}{Enzyme\ activity\ in\ control}$$

Statistical analysis**:** The log concentration and mortality regression were worked out by log probit technique following the procedure of Finney 1951 and employing the computer programme SPSS v. 16.0 software**.**

RESULTS

Data presented in Table 3 revealed that PBO along with chlorantraniliprole recorded higher synergistic ratio (SR) of 3.50 followed by DEM with (3.30) and N-(Propagyloxy) phthalimide (0.76). Among the rest, *Pongamia* oil recorded higher synergistic ratio of 0.55 whereas *Callophyllum inophyllum* oil it was 0. 50 and hydroquinone had the lowest synergistic ratio of 0.423. In case of Flubendiamide, the synergistic ratio for PBO, DEM and N-(Propargyloxy) phthalimide were found as 2.31, 1.50 and 1.03 respectively while the other 3 synergists had recorded the value of 0.72, 0.20 and 0. 28. The fiducial limits, chi square values and probit regression lines for all the six synergists are mentioned in Table 3 for the Bhubaneswar population. For profenphos, highest synergistic ratio was recorded for PBO i.e., 4.70 followed by DEM 4.50 and N-(Propagyloxy) phthalimide occupied the third position of 4.16. Among the rest, *Pongamia* oil recorded higher synergistic ratio of 1.63 whereas hydroquinone had the synergistic ratio of 0.65 and *Callophyllum inophyllum* was 0. 70. So profenophos, the organophosphate insecticide recorded higher synergestic ratio towards all the tested chemicals than both the diamides insecticides.

Data pertaining to the synergistic study of same chemicals in Angul population are presented in Table Chlorantranileprol the dicarboxylic acid monoamie derivative recorded highest synergistic ratio with PBO (10.25) followed by DEM i.e. 5.11

Table 3. Effect of studied synergists on efficacy and toxicity of different insecticides on 3rd instar larvae of *L. orbonalis* collected from Bhubaneswar during *Kharif* 2021-2022.

Insecticide	Synergist	Dose of synergist used (µg/Larvae)	LD_{50} of insecticide & synergist (ng/Larvae)	SR ratio	Chi square value	Slope±SE	Regression equation	Fiducial limit	
Flubendiamide	Nil	-	0.14	-	0.415	0.881+0.083	Y=1.46+0.38x	0.051	0.751
	PBO	10	0.16	2.312	6.460	0.706±0.090	Y=2.20+0.46x	0.265	0.619
	DEM	20	0.14	1.500	6.241	0.737±0.091	Y=2.33+0.48x	0.292	0.690
	Hydroquinone	15	0.72	0.201	2.720	0.736±0.088	Y=1.34+0.32x	0.151	0.495
	N-(Propagyloxy) phthalimide	10	0.20	1.038	4.394	0.697±0.088	Y=1.52+0.36x	0.191	0.537
	Pongmia oil	10	0.29	0.724	3.650	0.733±0.088	Y=1.6+0.37x	0.196	0.542
	Callophyllum inophyllum oil	40	7.5	0.280	1.748	0.752±0.087	Y=1.13+0.27x	0.103	0.443
Chlorantraniliprole	Nil	-	0.1	-	0.846	0.878±0.086	Y=1.98+0.5x	0.046	0.258
	PBO	10	0.02	3.500	4.690	0.747±0.091	Y=2.37+0.44x	0.242	0.601
	DEM	20	0.03	3.360	4.150	0.724±0.090	Y=2.06+0.36x	0.187	0.540
	Hydroquinone	15	0.23	0.423	4.350	0.725±0.089	Y=1.85+0.39x	0.211	0.558
	N-(Propagyloxy) phthalimide	10	0.13	0.769	4.110	0.704±0.088	Y=1.73+0.35x	0.173	0.518
	Pongmia oil	10	0.18	0.555	2.790	0.757±0.088	Y=1.67+0.35x	0.174	0.518
	Callophyllum inophyllum oil	40	0.19	0.504	3.560	0.717±0.088	Y=1.49+0.35x	0.172	0.517
Profenophos	Nil	-	1	-	0.456	0.879±0.088	Y=1.22+0.4x	0.251	9.011
	PBO	10	0.21	4.700	2.490	0.740±0.087	Y=1.41+0.31x	0.136	0.477
	DEM	20	0.22	4.500	3.090	0.727±0.087	Y=1.53+0.33x	0.155	0.497
	Hydroquinone	15	0.15	0.650	2.040	0.739±0.087	Y=1.19+0.28x	0.109	0.449
	N-(Propagyloxy) phthalimide	10	0.24	4.160	2.600	0.724±0.086	Y=1.09+0.24x	0.138	0.479
	Pongmia oil	10	0.61	1.630	3.750	0.729±0.089	Y=1.59+0.38x	0.202	0.551
	Callophyllum inophyllum oil	40	1.41	0.700	2.560	0.740±0.088	Y=1.24+0.32x	0.151	0.497

and for N-(Propagyloxy) phthalimide it was 4.09. Rest three i.e., *Pongamia* oil, *Callophyllum inophyllum* oil and hydroquinone recorded SR value of 1.63, 0.67 and 0.43, respectively. The SR values for PBO, DEM and N-(Propagyloxy) phthalimide recorded in this population towards Flubendiamide were 25.00, 12.50 and 8.33 respectively whereas *Pongamia* oil recorded the value as 4.00 and the rest two could not perform satisfactorily. Values for other characters and the regression equation towards flubendiamide are presented in Table 3. Profenophos synergism with PBO, DEM and N-(Propagyloxy) phthalimide were found as 33.33,31.75 and 24.440Suprising result was obtained for *Pongamia* oil where the SR was 16.000. In *Callophyllum inophyllum* oil and hydroquinone the SR value was 5.19 and 4.08 respectively.

The synergistic activity of PBO towards several group of insecticides has been well documented by several earlier workers in different insect species (Olusegun –Joseph *et al.*, 2019). MFOs are established detoxifying system in each organism including insects for enhancing detoxification and metabolism occurs via oxidation, thus considered as an important tool in development of resistance (Feyereisen, R., 2012). In the present study Angul population recorded more SR to all the chemicals than Bhubaneswar population suggesting the population are more resistant towards the tested insecticides than Bhubaneswar population.

The major components i.e., Cytochrome P_{450}, Cytochrome P_{420} and Cytochrome b_5 were determined in 3rd instar larvae of this pest from both the population has been presented in Table 4. In the present context Cytochrome P_{450} was found as the major component and supposed to be responsible for detoxification with major share in development of resistance. Data in Table 5. revealed that in Bhubaneswar population Chlorantraniliprole applied at LD_{30} dose recorded enhanced activity of MFOs (b_5) to the tune of 1.07 times, 1.48 times and 1.47 times after 24, 48 and 72 hrs. of treatment respectively than untreated insects. The value recorded for cytochrome P_{450} after the same intervals were 2.84, 2.87 and 2.84. For cytochrome P_{420}, the activity ratio recorded were 1.07, 3.22 and 2.46 only. Sub-lethal dose application of flubendiamide recorded less enhanced activity ratio of 0.82, 1.22 and 1.23 after different duration of application of insecticides. For Cytochrome b_5 and cytochrome P_{450} the activity ratio after 24hrs was 0.82, and3.42, after 48 hrs. 3.70 and1.22 and after

Table 4. Effect of studied synergists on toxicity of different insecticides on 3rd instar larvae of *L. orbonalis* collected from Angul during *Kharif* 2021-22.

Insecticide	Synergist	Dose of synergist (μg/Larvae)	LD_{50} of insecticide & synergist (ng/larvae)	SR ratio	Chi square	Slope±SE	Regression equation	Fiducial limit	
Flubendiamide	Nil	-	1.04	-	0.25	0.987±0.087	Y=1.08+0.36x	0.267	4.68
	PBO	10	0.04	25	3.84	0.805±0.089	Y=1.83+0.34x	0.158	0.506
	DEM	20	0.08	12.5	3.49	0.833±0.088	Y=1.80+0.35x	0.174	0.52
	Hydroquinone	15	2.44	4.09	3.15	0.829±0.090	Y=1.27+0.35x	0.179	0.532
	N-(Propagyloxy) phthalimide	10	0.12	8.333	3.045	0.812±0.087	Y=1.51+0.31x	0.133	0.474
	Pongmia oil	10	0.25	4	2.706	0.832±0.087	Y=1.43+0.31x	0.139	0.48
	Callophyllum inopyllum oil	40	1.55	0.645	3.79	0.833±0.090	Y=1.49+0.39x	0.219	0.574
Chlorantraniliprole	Nil	-	0.409	-	0.568	0.978±0.086	Y=1.40+0.41x	0.148	2.64
	PBO	10	0.04	10.22	4.195	0.797±0.089	Y=1.85+0.35x	0.162	0.51
	DEM	20	0.08	5.112	4.071	0.823±0.089	Y=1.91+0.38x	0.191	0.538
	Hydroquinone	15	0.93	0.439	3.27	0.834±0.089	Y=1.43+0.35x	0.183	0.53
	N-(Propagyloxy) phthalimide	10	0.1	4.09	2.708	0.857±0.088	Y=1.47+0.34x	0.165	0.509
	Pongmia oil	10	0.25	1.636	4.774	0.808±0.089	Y=1.77+0.38x	0.207	0.554
	Callophyllum inophyllum oil	40	0.6	0.675	3.15	0.827±0.08	Y=1.35+0.35x	0.168	0.515
Profenophos	Nil	-	4.01	-	0.297	0.982±0.091	Y=0.85+0.35x	1.12	31.44
	PBO	10	0.12	33.33	4.19	0.823±0.089	Y=1.88+0.38x	0.202	0.549
	DEM	20	0.11	31.75	4.05	0.822±0.088	Y=1.82+0.37x	0.189	0.536
	Hydroquinone	15	0.98	4.081	3.09	0.812±0.088	Y=1.27+0.32x	0.147	0.491
	N-(Propagyloxy) phthalimide	10	0.16	24.44	2.24	0.836±0.087	Y=1.44+0.29x	0.113	0.453
	Pongmia oil	10	0.25	16	2.71	0.832±0.087	Y=1.43+0.31x	0.139	0.48
	Callophyllum inopyllum oil	40	0.77	5.19	2.47	0.866±0.088	Y=1.42+0.35x	0.173	0.519

Table 5. Effect of sub lethal doses of the studied insecticides on MFOs activity in population collected from Bhubaneswar during *Kharif* 2022.

Name of the insecticide	Components of MFOs Activities of MFOs after hrs of Treatment (μMol/mg protein/min)						
		24hrs	Activity ratio	48hrs	Activity ratio	72hrs	Activity ratio
Chlorantraniliprole (LD_{30}= 0.013)	Cytochrome b_5	2.05±0.21	1.07	3. 11±0.22	1.48	3.91±0.23	1.47
	Cytochrome P_{450}	3.78±0.25	2.84	5.67±0.24	2.87	7.32±0.24	2.84
	Cytochrome P_{420}	4.34±0.26	1.07	6.35±0.26	3.22	6.12±0.25	2.46
Flubendiamide (LD_{30}= 0.011)	Cytochrome b_5	1.58±0.21	0.82	2.58±0.23	1.22	3.27±0.24	1.23
	Cytochrome P_{450}	4.56±0.23	3.42	7.29±0.25	3.70	4.45±0.26	1.73
	Cytochrome P_{420}	4.24±0.25	3.31	9.25±0.26	4.34	5.09±0.27	2.05
Profenophos (LD_{30}= 0.052)	Cytochrome b_5	1.89±0.22	0.99	3.21±0.24	1.52	4.40±0.24	1.66
	Cytochrome P_{450}	3.78±0.24	2.84	5.90±0.25	2.99	4.12±0.26	1.60
	Cytochrome P_{420}	4.79±0.26	3.74	6.21±0.26	2.91	5.71±0.27	2.30
Control	Cytochrome b_5	1.91±0.19	-	2.10±0.20	-	2.65±0.21	-
	Cytochrome P_{450}	1.33±0.20	-	1.97±0.22	-	2.57±0.22	-
	Cytochrome P_{420}	1.28±0.21	-	2.13±0.23	-	2.48±0.24	-

72 hrs.it was 1.23and 1.73. For cytochrome P_{420}, the, activity ratio was 3.31, 4.34 and 2.05 after 24 hrs. 48 and 72 hrs. respectively. Profenophos application at LD_{30} dose induces cytochrome b5 after 24hrs with activity ratio value of 0.99, whereas after 48 hrs. it was 1.52 and 1.66 after 72hrs. For Cytochrome P_{450} after 24 hrs. of application it was 2.84, after 48hrs the value was 2.99 and after 72hrs it was 1.60. For Cytochrome P_{420} activity ratio after 24 hrs. was 3.74, after 48hrs it was 2.91 and after 72hrs it was 2.30.

Microsomal oxidases were determined directly from larvae collected from fields during *kharif* from both the places. Angul population recorded higher titters of all the three

Table 6. Effect of sub-lethal doses of the studied insecticides on mfos activities in population collected from Angul during *Kharif* 2022.

Name of the insecticide	Components of MFOs	Activity of MFO after hrs of treatment (µM/mg protein/min)					
		24h	Activity ratio (24h)	48h	Activity ratio (48h)	72h	Activity ratio (72h)
Chlorantraniliprole	Cytochrome b_5	2.89±0.23	1.48	4.12±0.24	1.91	5.58±0.26	2.05
(LD_{30} =0.029)	Cytochrome P_{450}	5.88±0.24	3.19	7.58±0.26	3.47	8.27±0.28	3.48
	Cytochrome P_{420}	5.98±0.27	3.01	8.11±0.28	3.66	8.92±0.30	3.68
Flubendiamide	Cytochrome b_5	2.29±0.22	1.17	4.08±0.23	1.88	5.19±0.23	1.91
(LD_{30}=0.047)	Cytochrome P_{450}	7.36±0.24	4.00	8.99±0.25	4.12	9.79±0.26	4.13
	Cytochrome P_{420}	7.39±0.26	3.73	9.75±0.26	4.41	9.91±0.27	4.09
Profenophos	Cytochrome b_5	2.55±0.23	1.31	4.36±0.24	2.02	5.66±0.25	2.08
(LD_{30} =0.166)	Cytochrome P_{450}	5.71±0.25	3.10	7.59±0.26	3.48	8.28±0.28	3.5
	Cytochrome P_{420}	6.23±0.26	3.14	7.38±0.28	3.33	7.99±0.30	3.3
CONTROL	Cytochrome b_5	1.95 ±0.21	-	2.16±0.23	-	2.71 ±0.24	-
	Cytochrome P_{450}	1.84 ±0.22	-	2.18 ±0.25	-	2.37 ±0.26	-
	Cytochrome P_{420}	1.98 ±0.23	-	2.21 ±0.26	-	2.42 ±0.27	-

studied components of MFOs (Cytochrome b_5, Cytochrome P_{450} and Cytochrome P_{420}) and the same trend was repeated after different hrs of treatment (Table 6).

DISCUSSION

The literature pertaining to investigated subject of *L. orbonalis* is quite migre because of the typical boring feeding habit of this pest In Angul population induction of all three components was found up to 72hrs of application but Bhubaneswar population recorded maximum activity up to 48hrs.after which there is a downfall in activity which shows that application of under dose will protect the pest against the tested insecticides up to 72hrs in Angul population and 48hrs in Bhubaneswar population. At Angul the crop is exposed to all type of conventional and new insecticides after each plucking i.e., 6-7 days interval but the Bhubaneswar population is exposed to mostly diamides, emamectin etc., after 12-15 days interval. But in the present study the genetic basis of differential prolongation of MFOs induction has not been studied. The present finding corroborate the finding of Shehawy and Alshehri (2015) who observed a significant elevation in MFO activity in bean aphids treated with Imidacloprid and Pirimicarb. In the field colony of aphids, they recorded more activity ratio than the susceptible lab strain. Involvement of Cytochrome P_{450} mono-oxygenase in resistant mosquitos was reported by Kasai *et al.*, 1998 and in saw toothed grain beetle by Rose *et al.* (1986). Pottelberg *et al.*, (2008) evaluated six Cytochrome P_{450} inducers in insects where peak induction was found at 48hrs. This was reported for red spider mite *Tetranychus urticae.* Here, it is clear that PBO is acting as a potential synergist to all the tested insecticides in both the shared population which obviously says that MFO system plays a major role in detoxification, via oxidation followed by Glutathion conjugation as DEM is recording the second best SR values. Esterases although plays a little role, still important. Among the botanicals *Pongamia* oil is significantly synergising all the 3 tested insecticides in both the population although its exact mode of action is yet to be established. Given that DEM is registering the highest SR values, it is obvious that PBO is acting as a potential synergist to all of the tested insecticides in both populations. This indicates that the MFO system plays a significant role in detoxification through oxidation followed by glutathion conjugation. Despite having a minor influence, esterase's nonetheless came in second. Pongamia oil is one of the botanicals that considerably synergizes all three of the studied insecticides in both populations, however its precise route of action is still unknown.

***Conclusion*:** In conclusion, this study emphasizes the significance of mixed function oxidases in the process of detoxifying insecticides and xenobiotics, specifically in the context of *L. orbonalis* infestation in brinjal crops. Future researchers should consider the evolving challenge of resistant strains to newly developed insecticides, as it hinders effective population control of this pest. The study evaluates a range of chemicals, including antioxidants and plant oils, and identifies a promising combination of pipernyl butoxide, diethyl malate, and pongamia oil with chlorantraniliprole, flubendiamide, and profenophos. Notably, the population from Angul demonstrates higher synergistic ratios compared to Bhubaneswar. Moreover, pongamia oil showcases a significant synergistic effect on all tested insecticides. These discoveries provide valuable insights for the development of successful strategies to combat *L. orbonalis* infestation and improve pest management in brinjal crops.

***Authors contributions statement*:** M.K. Tripathy, S. Khuntia designed, completed the experiments; S.S. Dash, S. Quadri

prepared the draft; N. Singh, M.K. Tripathy reviewed and finalized the draft.

***Conflict of interest*: The authors declare no conflict of interest.**

Acknowledgement: The authors thank and appreciate the cooperation of Dept. of Entomology, College of Agriculture, OUAT, Bhubaneswar, Odisha, India.

Funding: The authors acknowledge Dept. of Entomology, College of Agriculture, OUAT, Bhubaneswar, Odisha, India for providing research funding to conduct this experiment.

Ethical statement: This research on brinjal shoot and fruit borer followed ethical guidelines and received necessary approvals to ensure humane treatment of subjects and adherence to animal welfare principles.

Availability of data and material: We declare that the submitted manuscript is our work, which has not been published before and is not currently being considered for publications elsewhere.

Code availability: Not Applicable

Consent to participate: All authors participated in this research study.

Consent for publication: All authors' submitted consent to publish this research article in JGIAS.

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